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(54) Title: PRISM DESIGN FOR OPTICAL SCANNING APPLICATIONS
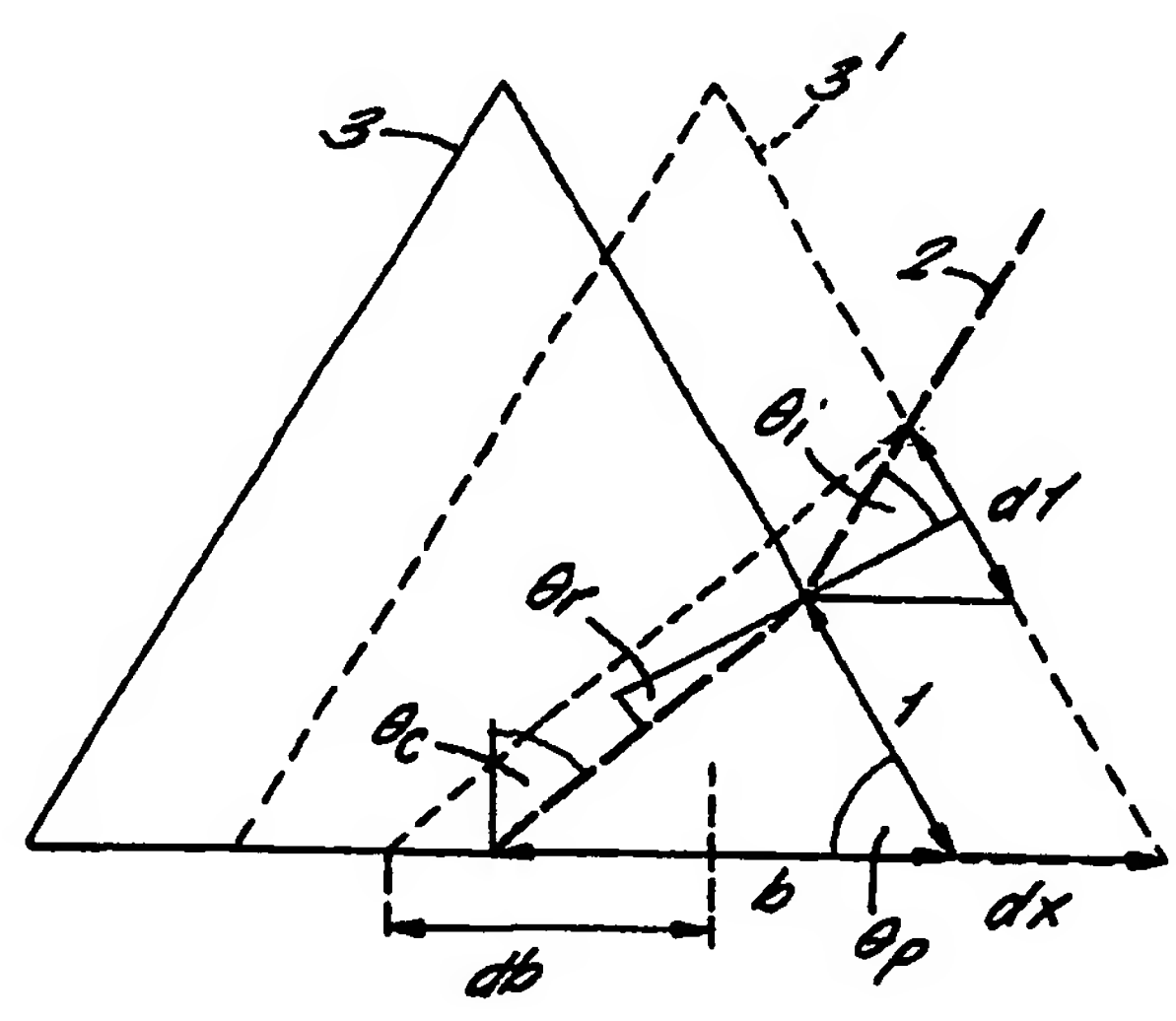
(57) Abstract: There is disclosed a prism for use in scanning applications such as total internal reflection microscopy in which the prism is translated relative to an incident light beam. A geometry is disclosed which cancels walk of the beam footprint at the base of the prism.

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PRISM DESIGN FOR OPTICAL SCANNING APPLICATIONS·

The present invention relates to scanning
applications in which a sample, together with an angular
5   optic ie a triangular or trapezoidal prism, are scanned in
a fixed laboratory frame of reference. The fixed
laboratory frame is defined by a fixed light source.
generating a fixed light beam and a detector. The angular
optic couples the light beam to the base interface of the
10   angular optic where the sample is located, such that the
beam is incident on the base interface at an off normal
angle. In such applications it is desirable that the
intercept of the light beam at the base interface of the
angular optic (the "footprint") remains stationary in the
15   laboratory frame as the optic and sample are scanned so
that there is no loss of image integrity at the detector.

One example of such an application is Total Internal
Reflection Microscopy, which is a technique for observing
20   samples illuminated by an evanescent wave. Total internal
reflection occurs when a beam of light travelling through
a very dense medium such as glass encounters an interface
with a less dense medium such as air or water, at an angle
to the normal which is greater than the critical angle for
25   the interface. The critical angle for a glass/water
interface is given by Fresnel's Law of Refraction as:

$$\theta_c = \sin^{-1}(n_{water}/n_{glass})$$

30   At angles greater than the critical angle, when total
internal reflection takes place, an electric field
component of the light penetrates through the interface
into the water as an evanescent wave. The evanescent wave
has the same wavelength as the incident beam but penetrates
35   only a very short distance into the water, typically no
more than 1 μm. The evanescent wave decays exponentially
from the interface into the water with a characteristic

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penetration depth dependent on the wavelength and angle of incidence of the totally internally reflected light.

In Total Internal Reflection Fluorescence Microscopy,
5   fluorophores may be excited by the light in the evanescent
field if they are close to the glass/water interface, but
fluorophores further away in the bulk of the solution will
not be excited. The result is that images with very low
background fluorescence are obtained. Figure 1 shows a
10   typical instrument set up used in Total Internal Reflection
Fluorescence Microscopy. A sample is placed such that it
is located directly on the interface of the base of a light
coupling optic or dispersion prism. Alternatively, a
glass slide may be optically matched to the prism, and the
15   sample located on the base of the slide. Total internal
reflection   then   occurs   at   the   base   of   the   slide.
Typically, the objective lens and external light source are
fixed in the lab frame and the sample on which the light
coupling optic or prism is fixed is scanned in a plane
20   perpendicular   to   the   objective   lens   axis.   The   prism
therefore moves relative to the objective lens and the
light source. Conventionally a 45° or 60° dispersion prism
is used, but to obtain light beams incident on the base of
the prism at angles close to and greater than the critical
25   angle, the light must usually be incident on the input face
of the prism at off normal angles of incidence to achieve
refraction of the beam at the air/glass interface. The
deviation of the beam causes the reflection footprint at
the base of the prism to walk db as the prism is translated
30   dx towards or away from the light source. In a limiting
case light propagating parallel to the prism base will be
refracted such that the footprint at the prism base moves
equally and in the same direction as the prism ($db/dx = 0$).
In this case the footprint moves dx in the lab frame and
35   the illuminated area moves rapidly away from the imaging
lens as the sample is scanned.

It is an object of the present invention to obtain a footprint which is static in the laboratory frame of reference, defined by the objective lens and light source, such that the area illuminated at the glass/aqueous interface does not move away from the optical axis of the lens as the sample and prism are scanned.

According to the present invention, a scanning apparatus comprises:-

a light source for generating a light beam; and

an angular prism coupled to a sample at a base interface;

characterised in that the base angle θ_p of the prism satisfies the equations:-

$$\frac{(\cos^2\theta_c - \sin^2\theta_p)\ (\tan\theta_i\ \sin\theta_p + \cos\theta_p)}{\cos\theta_c\ \cos(\theta_p +/- \theta_c)} = 1$$

and

$$n_i\ \sin\theta_i = n_p\ \sin\ (\theta_p - \theta_c)$$

wherein θ_c is the coupling angle required for light incident at the base interface of the prism, θ_i is the incident angle of the light beam on the prism, n_i is the refractive index of the medium at the interface where the light beam enters the prism and n_p is the refractive index of the prism.

According to the present invention, a scanning method comprises the steps of:-

generating a light beam;

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providing an angular prism in the path of the light beam, the prism being coupled to a sample at a base interface; and

5   moving the prism and sample relative to the light beam;

characterised in that the base angle θ_p of the prism satisfies the equations:-

10

$$\frac{(\cos^2\theta_c - \sin^2\theta_p)\ (\tan\theta_i\ \sin\theta_p + \cos\theta_p)}{\cos\theta_c\ \cos(\theta_p +/- \theta_c)} = 1$$

and

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$$n_i\ \sin\theta_i = n_p\ \sin\ (\theta_p - \theta_c)$$

wherein θ_c is the coupling angle required for light incident at the interface at the base interface of the
20   prism, θ_i is the incident angle of the light beam on the prism, n_i is the refractive index of the medium at the interface where the light beam enters the prism and n_p is the refractive index of the prism.

25   It has been found that, if the prism satisfies the above criteria, a solution exists where the footprint of the light beam on the base of the prism walks in an equal and opposite direction to the prism's translation in the lab frame i.e. $db = -dx$. Therefore, the point where the
30   light incident to the prism intercepts the prism base is fixed in the lab frame. This results in scanning of the sample without movement of the footprint with respect to a detector fixed in the lab frame and hence no loss of image integrity.

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A solution is found wherein $\theta_p = \theta_c$ and $\theta_i = 0$.

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Preferably, the apparatus comprises a total internal reflection microscopy apparatus and includes means for detecting interaction of the sample at the base of the prism or optically matched slide with an evanescent wave formed by total internal reflection of the light beam at the base of the prism or at the base of a slide which is optically matched to the prism. Preferably, the method according to the present invention includes the step of detecting interaction of a sample at the base of the prism with an evanescent wave formed by total internal reflection of the light beam at the base of the prism.

For light entering the prism from air, $n_i = 1$, and therefore

$$\sin \theta_i = n_p \sin (\theta_p - \theta_c).$$

For total internal reflection to occur at the base interface θ_c must be greater than or equal to the critical angle for the interface ie

$$\theta_c \geq \sin^{-1} (n_s/n_p)$$

Wherein n_s is the refractive index of the sample medium.

In total internal reflection microscopy, it is preferable that the coupling angle is greater than but close to the critical angle as this maximises the penetration of the evanescent wave into the sample medium. It is generally preferable that θ_c is slightly above the critical angle because, although penetration of the evanescent wave is at a maximum at the critical angle, there will be a spread of angles within the beam and, to ensure total internal reflection of the entire beam it is preferable to have θ_c slightly above the critical angle.

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This also accommodates minor variations in the refractive indices of the interfacial media.

For a quartz/water interface at the base of the prism, where $n_p=1.46$, the critical angle of the base interface is 66°. A preferred value for θ_c would be 68°. A unique solution is found wherein $\theta_i = 0$, and $\theta_p = 68°$.

A preferred embodiment of the present invention will now be described with reference to the accompanying drawings; in which:-

Figure 1 shows an apparatus for Total Internal Reflection Fluorescence Microscopy;

Figure 2 is a schematic showing the total internal reflection footprint walking with the displacement of the prism; and,

Figure 3 is a graph of db/dx against prism angle for a quartz/water base interface and a coupling angle of 68°.

Figure 1 illustrates an apparatus for Total Internal Reflection Fluorescence Microscopy having a light source 1 which generates a beam 2 which is incident on a prism 3 and is totally internally reflected at the base interface of the prism 3. A sample is positioned adjacent to a base interface of the prism 3 and the evanescent wave interacts with the sample, producing fluorescence. The fluorescence passes through an objective lens 4 and is directed towards a CCD camera 5 by a mirror 6, passing through a filter 7.

As shown in Figure 2, the light beam 2 is incident on the prism 3 at θ_i to the normal, is refracted as it enters the prism at θ_r to the normal and is incident on the base of the prism 3 at a coupling angle θ_c, forming a footprint at the base interface. For total internal reflection to

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occur, θ_c must be at least the critical angle for the base interface. As the prism moves dx in the lab frame to position 3', the footprint walks db in the prism frame.

5   The magnitude of the differential dl/db may be derived as follows:-

Sine rule

$$\frac{l}{\sin(90-\theta_c)} = \frac{r}{\sin\theta_p} \quad \text{or} \quad r\cos\theta_c = l\sin\theta_p \quad (1)$$

10   Cosine rule

$$r^2 = b^2 + l^2 - 2bl\cos\theta_p \quad\quad\quad (2)$$

(1) in (2)

$$l^2\sin^2\theta_p = \cos^2\theta_c(b^2 + l^2 - 2bl\cos\theta_p)$$

15   quadratic in l

$$l^2(\cos^2\theta_c - \sin^2\theta_p) - 2bl\cos\theta_p\cos^2\theta_c + b^2\cos^2\theta_c = 0$$

solution to which is

20

$$l = \frac{2b\cos\theta_p\cos^2\theta_c + \sqrt{4b^2\cos^4\theta_c\sin^2\theta_p - 4b^2\cos^2\theta_c(\cos^2\theta_c - \sin^2\theta_p)}}{2(\cos^2\theta_c - \sin^2\theta_p)}$$

expand contents of the square root

25

$$l = \frac{2b\cos\theta_p\cos^2\theta_c + \sqrt{4b^2\cos^4\theta_c\sin^2\theta_p - 4b^2\cos^4\theta_c + 4b^2\cos^2\theta_c\sin^2\theta_p}}{2(\cos^2\theta_c - \sin^2\theta_p)}$$

take out $4b^2\cos^2\theta_c$ common in the square root

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$$l = \frac{2b\cos\theta_p\cos^2\theta_c + 2b\cos\theta_c\sqrt{\cos^2\theta_c\sin^2\theta_p - \cos^4\theta_c + \sin^2\theta_p}}{2(\cos^2\theta_c - \sin^2\theta_p)}$$

factorizing gives

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$$l = \frac{b\cos\theta_c\left(\cos\theta_p\cos\theta_c + \sqrt{\cos^2\theta_c\cos^2\theta_p - \cos^2\theta_c + \sin^2\theta_p}\right)}{(\cos^2\theta_c - \sin^2\theta_p)}$$

differentiating w.r.t. b

40

$$\frac{dl}{db} = \frac{\cos\theta_c\left(\cos\theta_p\cos\theta_c + \sqrt{\cos^2\theta_c\cos^2\theta_p - \cos^2\theta_c + \sin^2\theta_p}\right)}{(\cos^2\theta_c - \sin^2\theta_p)}$$

further factorization gives

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$$\frac{dl}{db} = \frac{\cos\theta_c\left(\cos\theta_p\cos\theta_c + \sqrt{\cos^2\theta_c(\cos^2\theta_p - 1) + \sin^2\theta_p}\right)}{(\cos^2\theta_c - \sin^2\theta_p)}$$

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recognising $\cos^2\theta_p - 1 = -\sin^2\theta_p$ and factorising

$$\frac{dl}{db} = \frac{\cos\theta_c(\cos\theta_p\cos\theta_c + \sqrt{\sin^2\theta_p(1-\cos^2\theta_c)})}{(\cos^2\theta_c - \sin^2\theta_p)}$$

recognising $1-\cos^2\theta_c = \sin^2\theta_c$ and rooting the square

$$\frac{dl}{db} = \frac{\cos\theta_c(\cos\theta_p\cos\theta_c + \sin\theta_p\sin\theta_c)}{(\cos^2\theta_c - \sin^2\theta_p)}$$

using trigonometric identity the differential simplifies to

$$\frac{dl}{db} = \frac{\cos\theta_c\cos(\theta_p + \theta_c)}{(\cos^2\theta_c - \sin^2\theta_p)}$$

The magnitude of the differential dl/dx may be derived as follows:-

Sine rule $\qquad \dfrac{dl}{\sin(90-\theta_p+\theta_l)} = \dfrac{dx}{\sin(180-\theta_p-(90-\theta_p+\theta_l))}$

Simplifies to $\qquad dl\,\sin(90-\theta_l) = dx\,\sin(90-\theta_p+\theta_l)$

$$dl\,\cos\theta_l = dx\,\cos(\theta_p - \theta_l)$$

$$\frac{dl}{dx} = \frac{\cos\theta_p\cos\theta_l + \sin\theta_p\sin\theta_l}{\cos\theta_l}$$

$$\frac{dl}{dx} = \cos\theta_p + \tan\theta_l\sin\theta_p$$

Recognising that a translation of the prism dx in the positive x direction results in a displacement db of the footprint in the negative direction and by using the chain rule $\qquad -\dfrac{db}{dx} = \dfrac{dl}{dx} \times \dfrac{db}{dl}$

so $\qquad \dfrac{db}{dx} = \dfrac{-(\cos\theta_p + \tan\theta_l\sin\theta_p) \times (\cos^2\theta_c - \sin^2\theta_p)}{\cos\theta_c\cos(\theta_p \pm \theta_c)}$

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where $\theta_l = \sin^{-1}(n_p\sin(\theta_p\text{-}\theta_c)$ from Fresnel's equation, for light entering the prism from air, $(n_l = 1)$.

For the footprint to remain stationary in the lab frame, it must walk in an equal and opposite direction in the prism frame to the prism's translation in the lab frame, i.e. $db/dx = -1$.

In total internal reflection microscopy, it is preferred that the coupling angle is greater than but close to the critical angle as this maximises the penetration of the evanescent wave into the sample medium, and it is generally preferable that θ_c is slightly above the critical angle because, although penetration of the evanescent wave is at a maximum at the critical angle, there will be a spread of angles within the beam and, to ensure total internal reflection of the entire beam, it is preferable to have θ_c slightly above the critical angle.

For a quartz/water interface at the base of the prism, where $n_p=1.46$, the critical angle of the base interface is $66°$. A preferred value for θ_c would be $68°$. Figure 3 shows the degree of walking of the footprint with prism displacement as a function of the internal angle θ_p of the prism for $\theta_c = 68°$. The prism angle required for $db/dx = -1$ is $68°$ and the light will be incident normal to the input surface of the prism, i.e. $\theta_l = 0$.

CLAIMS

1.   A scanning apparatus comprising:-

a light source for generating a light beam; and

an angular prism coupled to a sample at a base. interface;

characterised in that the base angle θ_p of the prism satisfies the equations:-

$$\frac{(\cos^2\theta_c - \sin^2\theta_p)\ (\tan\theta_i\ \sin\theta_p + \cos\theta_p)}{\cos\theta_c\ \cos(\theta_p +/- \theta_c)} = 1$$

and

$$n_i\ \sin\theta_i = n_p\ \sin\ (\theta_p - \theta_c)$$

wherein θ_c is the coupling angle required for light incident at base interface of the prism, θ_i is the incident angle of the light beam on the prism, n_i is the refractive index of the medium at the interface where the light beam enters the prism and n_p is the refractive index of the prism.

2.   A scanning apparatus according to claim 1, wherein $\theta_p = \theta_c$ and $\theta_i = 0$.

3.   A total internal reflection microscopy apparatus according to claim 1 or 2, including means for detecting interaction of the sample at the base of the prism with an evanescent wave formed by total internal reflection of the light beam at the base of the prism.

4.   A total internal reflection microscopy apparatus according to claim 3, wherein

$$\theta_c \geq \sin^{-1}(n_s/n_p)$$

and wherein n_s is the refractive index of the sample medium.

5.   A scanning method comprising the steps of:-

generating a light beam;

providing an angular prism in the path of the light beam, the prism being coupled to a sample at a base interface; and

moving the prism and sample relative to the light beam;

characterised in that the base angle θ_p of the prism satisfies the equations:-

$$\frac{(\cos^2\theta_c - \sin^2\theta_p)(\tan\theta_i \sin\theta_p + \cos\theta_p)}{\cos\theta_c \cos(\theta_p +/- \theta_c)} = 1$$

and

$$n_i \sin\theta_i = n_p \sin(\theta_p - \theta_c)$$

wherein θ_c is the coupling angle required for light incident at the interface at the base interface of the prism, θ_i is the incident angle of the light beam on the prism, n_i is the refractive index of the medium at the interface where the light beam enters the prism and n_p is the refractive index of the prism.

6.   A method of total internal reflection microscopy according to claim 5, further comprising the step of detecting interaction of a sample at the base of the prism with an evanescent wave formed by total internal reflection of the light beam at the base of the prism.

FIG. 1.
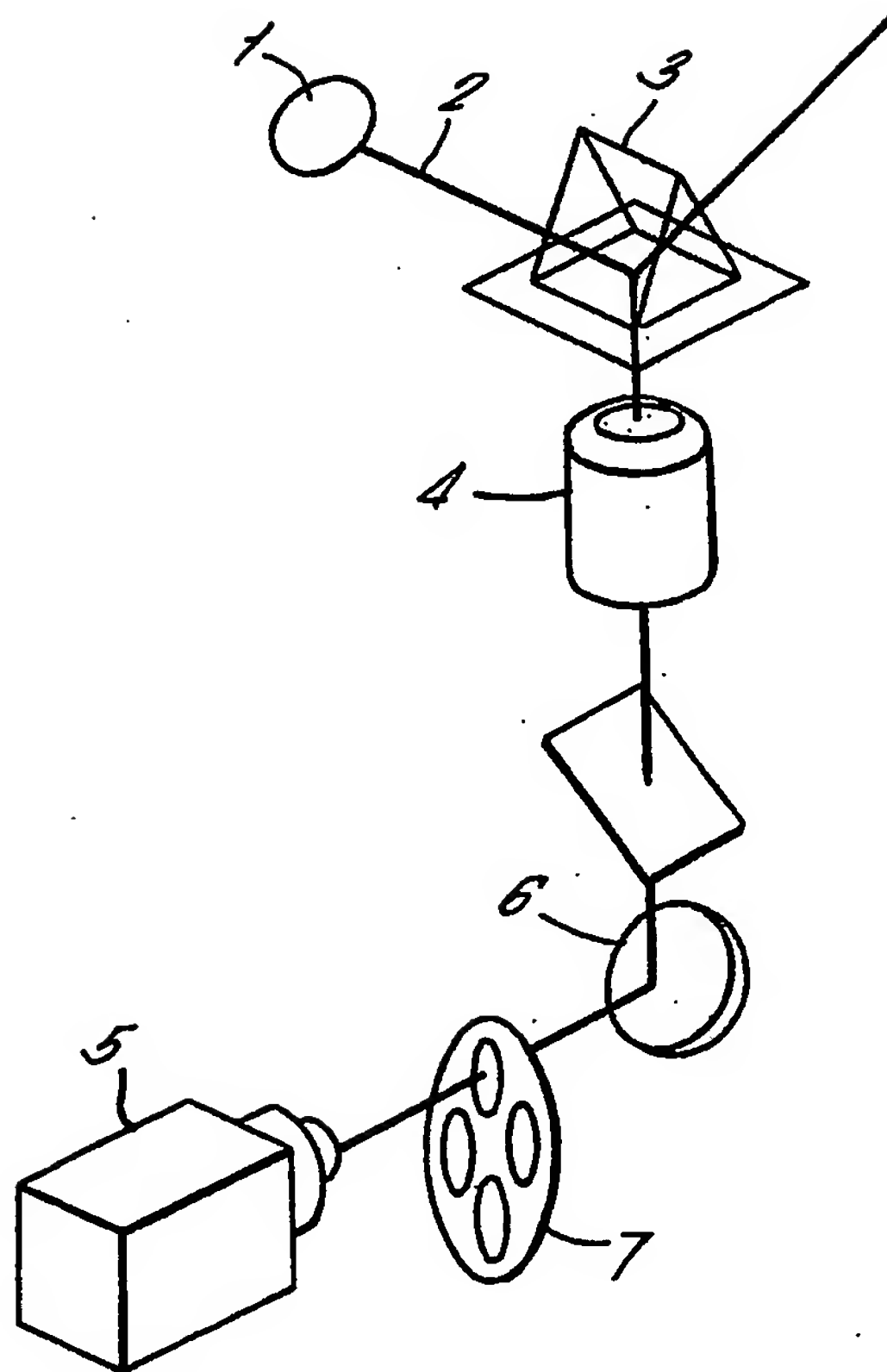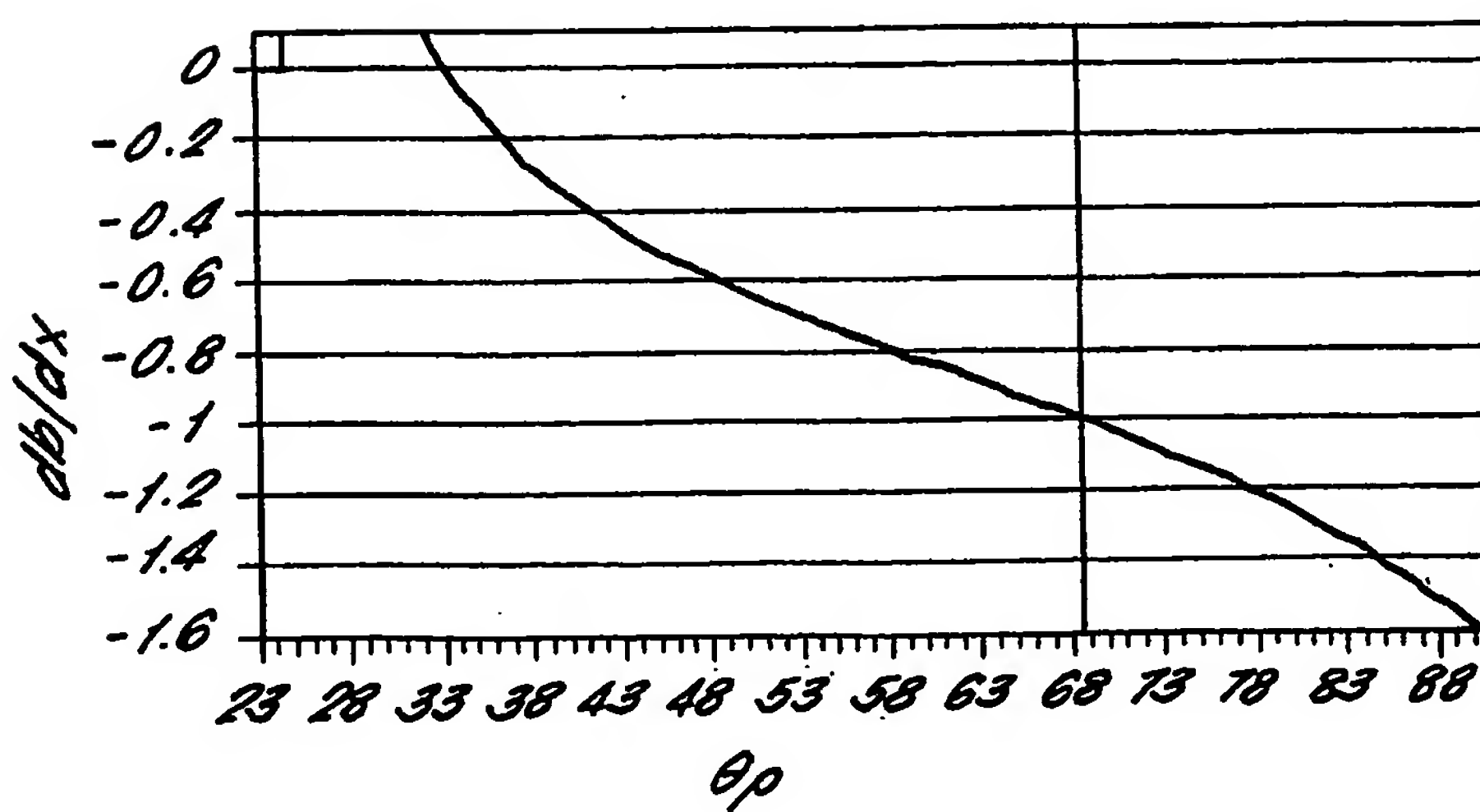
FIG.2.
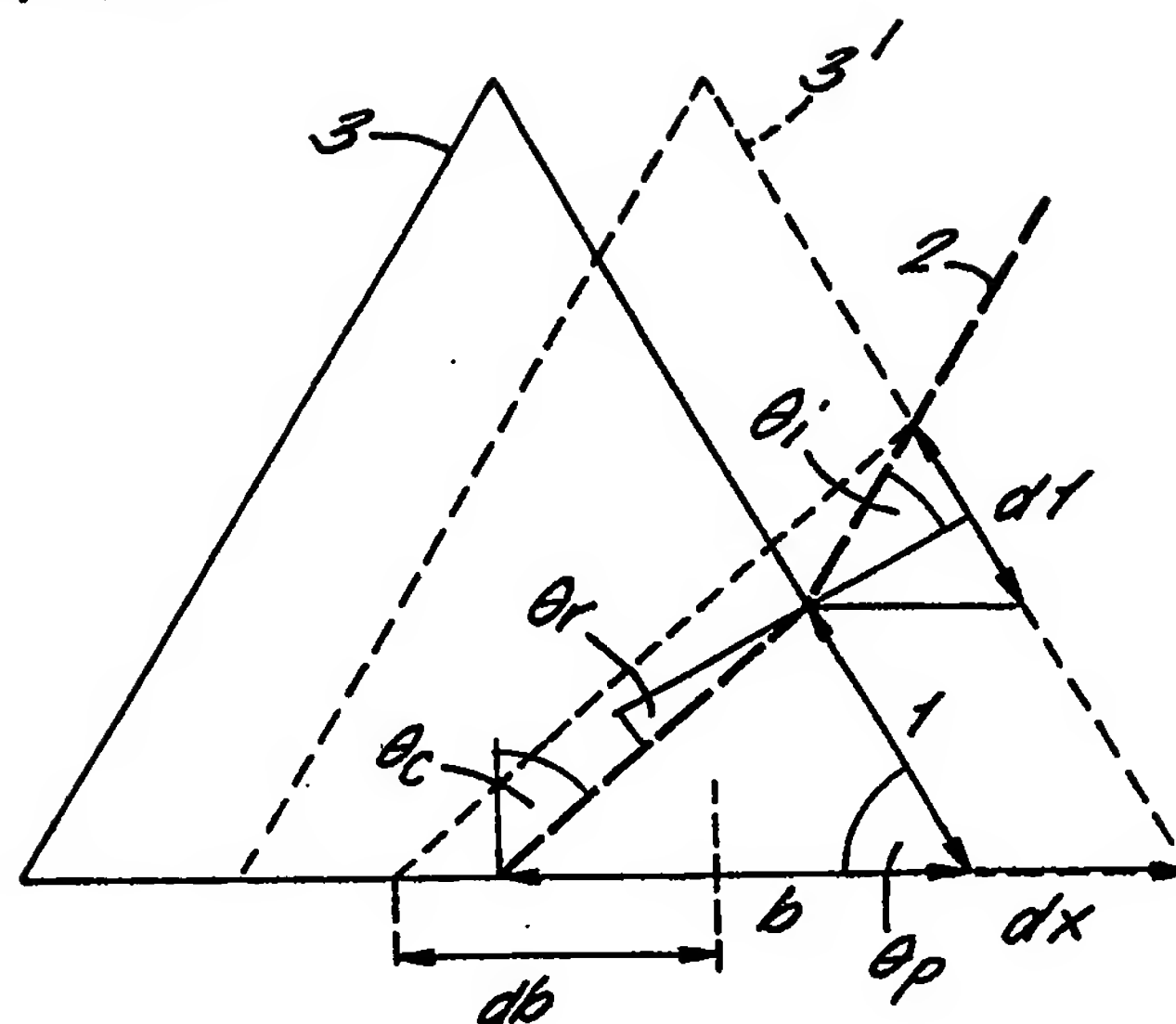
FIG. 3.

INTERNATIONAL SEARCH REPORT

PCT/GB 03/00154

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7   G02B21/00   G02B5/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7   G02B   G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6 327 238 B1 (NISHIWAKI SEIJI ET AL) 4 December 2001 (2001-12-04) abstract; figures 2-5 column 10, line 9 - line 27 ---	1-6
A	US 5 980 831 A (STONE JAMES J ET AL) 9 November 1999 (1999-11-09) the whole document ---	1-6
A	US 6 144 498 A (BRYARS BRETT J ET AL) 7 November 2000 (2000-11-07) example 1; table 1 ----	1-6

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

Date of the actual completion of the international search	Date of mailing of the international search report
18 March 2003	26/03/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Michel, A

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INTERNATIONAL SEARCH REPORT

Patent document cited in search report		Publication date	Patent family member(s)			Publication date
US 6327238	B1	04-12-2001	CN JP	1211790 11134701	A A	24-03-1999 21-05-1999
US 5980831	A	09-11-1999	US	6496636	B1	17-12-2002
US 6144498	A	07-11-2000	EP JP TW WO	1092170 2002517796 400439 9964915	A1 T B A1	18-04-2001 18-06-2002 01-08-2000 16-12-1999